A pH Dependent Switch Promotes β-Synuclein Fibril Formation via Glutamate Residues

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Running title: *Mildly Acidic pH Promotes Fibril Formation of β-Synuclein*

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Author contributions: GMM performed the NMR characterization, aggregation kinetics, experimental design, data analysis and manuscript preparation. MPO performed the computational modeling, chimera mutagenesis, and protein production. TBA performed AFM characterization, mutagenesis, and protein production. MKJ performed experimental design and assisted with chimera mutagenesis. SDK and JB were involved in experimental design, data analysis, and preparing the manuscript. All authors reviewed the results and approved the final version of the manuscript.

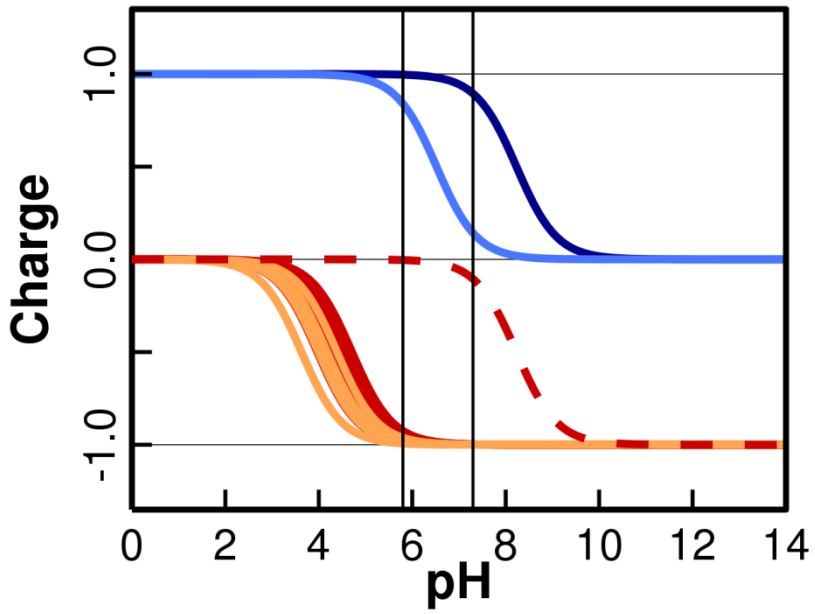

Figure S1: **An illustration of pH events in the experimental range for individual amino acids and αS domains in the experimental pH range.** Change of charge per pH unit is calculated from the Henderson-Hasselbach equation for the indicated amino acid sidechains with the pKa values measured for those measured in Croke, 2011(1),except for the amino terminus which is assumed to be pKa 8. The free-amino terminus is indicated in dark blue, histidine in light blue, glutamic acid in dark red and aspartic acid in orange. The dark red dashed line indicates a –COOH group that titrates with a pKa of 8. Vertical lines indicate the experimental pH range 5.8-7.3.

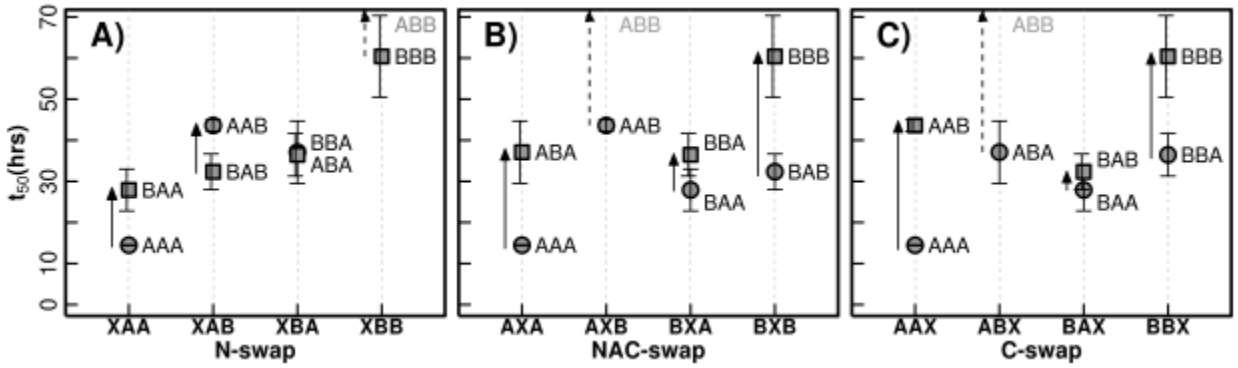

Figure S2: **A sequence comparison of the effect of individual αS/βS domains on the t_{50} of fibrillation.** Each panel examines the effect of having A or B in the X position as indicated by the x-axis labels under mildly acidic pH 5.8. The four possible combinations of N-terminal swaps are examined in A), NAC swaps in B) and C domain swaps in C). Samples which contain B in the X position are shown in square symbols. ABB is represented off scale, as it is the only non-ThT fluorescent sample at this pH and may be considered to have an indeterminably long t_{50}.

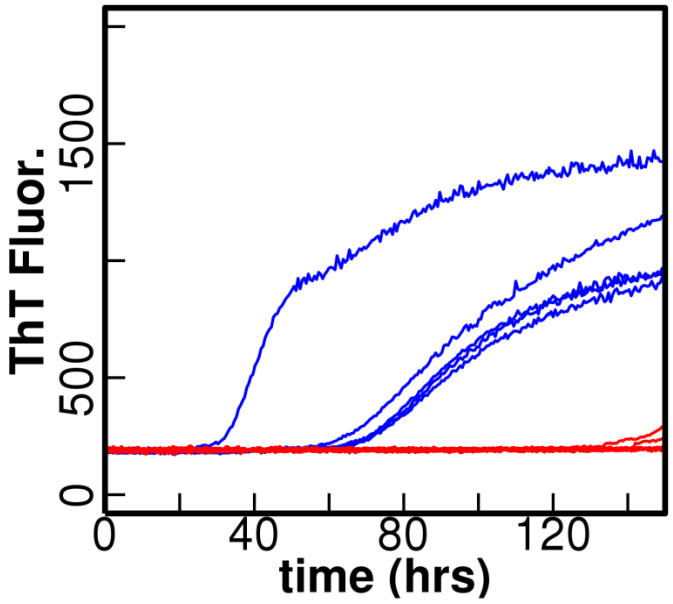

Figure S3: **ThT fluorescence of βS in alternate buffer at varying pH.** βS fibril formation, under the same conditions as described in Methods (600 rpm shaking, 37°C, ~1 mg/mL), also occurs in 20 mM sodium phosphate 100 mL salt as well as in the 20 mM MES 20 mM MOPS 100 mM NaCl buffer used in the main text. Low pH 5.8 is indicated in blue and high pH 7.3 in red. The raw fluorescence values are reported.

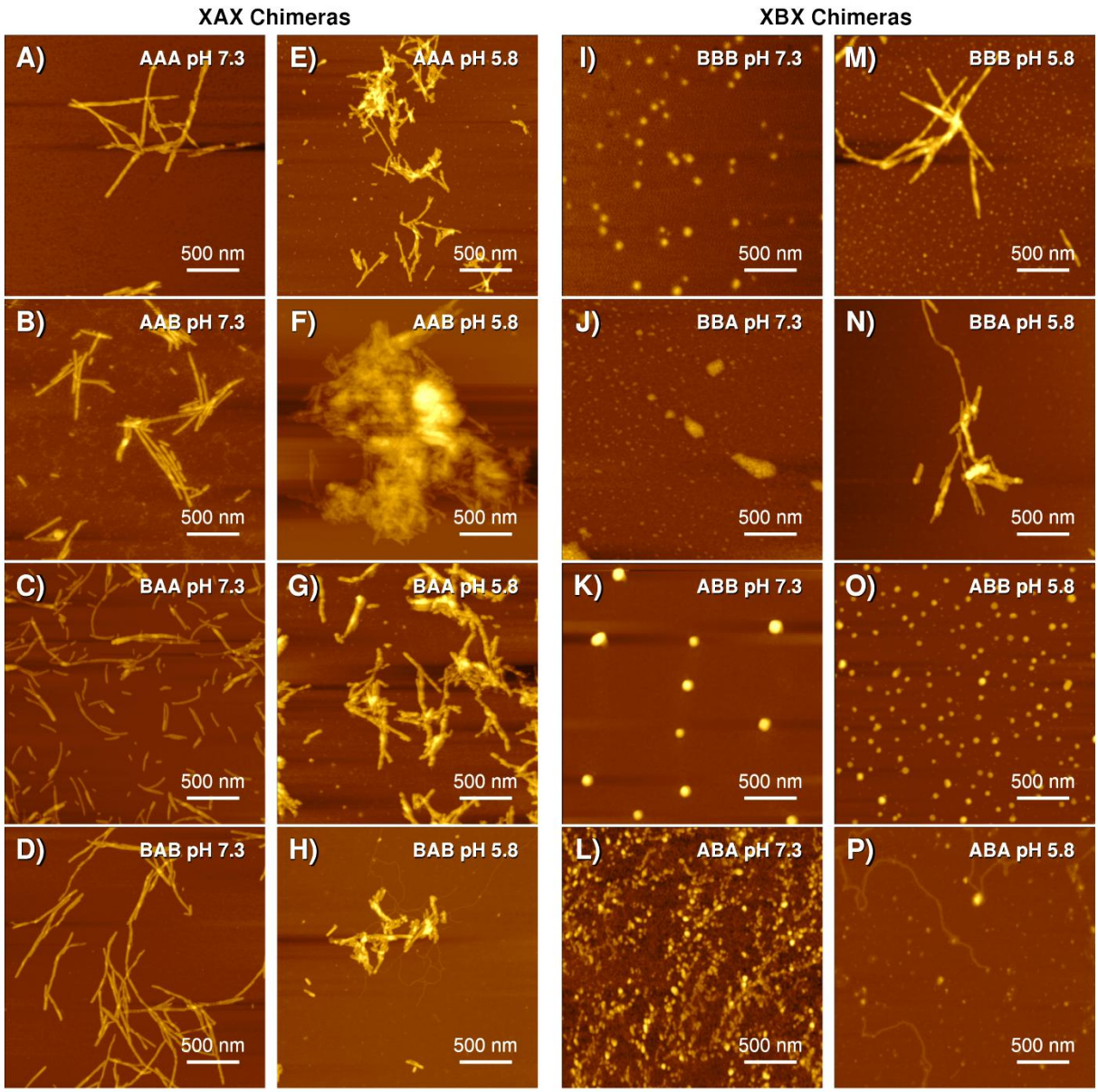

Figure S4: **pH dependent AFM imaging of αS/βS chimeras.** All chimeras were imaged from a sample of the 1 mg/mL samples at the plateau phase of fibrillation. In each case a 2.5 X 2.5 μm square was imaged, and the scale bar indicates a length of 500 nm. In A)-D) high pH 7.3 XAX chimeras are shown and in E) –H) low pH XAX chimeras are shown. Similarly in I)-L) and M)-P) are the low pH 5.8 and high pH 7.3 XBX chimeras, where X indicates either an A or B for an αS domain or a βS domain respectively. Panels A, E, I, M, N, O, and P, are the same as Fig

2D,2C,2F,2E,5C,5B, and 5D, respectively, and are reproduced here to facilitate an overall comparison of all constructs at both pH values.

Example Rosetta Scripts

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References

1. Croke, R. L., Patil, S. M., Quevreaux, J., Kendall, D. A., and Alexandrescu, A. T. (2011) NMR determination of pKa values in alpha-synuclein. *Protein Sci* **20**, 256-269